# THE MORPHOLOGY AND LIFE–HISTORY OF THE DIGENETIC TREMATODE, AZYGIA SEBAGO WARD, 1910 [1]

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The genus *Azygia* was erected by Looss (1899) to contain *Fasciola tereticollis* Rudolphi, 1802 (= *Fasciola lucii* Mueller, 1776, renamed). The worms were from the stomach of *Esox lucius*. According to Dawes (1946), this species, *Azygia lucii* (Mueller, 1776) Lühe, 1909, infects a number of different salmonid fishes, and other species of *Azygia* described from Europe are identical with it. The species has been reported in North America as *Distoma tereticolle* by Leidy (1851) from *Esox reticulatus;* by Stafford (1904) from *Esox lucius, Lota maculosa* and *Ameiurus nigricans;* and as *Azygia lucii* by Cooper (1915) from *Lucius lucius* (= *Esox lucius*), *Lucius masquinongy* (= *Esox masquinongy*), *Lioperca* sp., and immature specimens presumably of the same species were found in *Salvelinus namaycush* and *Micropterus dolomieu.*

Meanwhile, other species of *Azygia* were described in the United States and Canada. Leidy (1851) described *Distoma longum* on the basis of six specimens from the stomach of *Esox estor* Lesueur, 1818 (the American pike), collected near Cleveland, Ohio, and received from Professor Spencer F. Baird. The worms measured 30 to 76 mm. (3 inches) in length and as much as 1.6 mm. in breadth; the maximum diameter of the oral sucker was 1.27 mm. and of the acetabulum 1.06 mm. Measurements given by Leidy for specimens from the stomach of *E. reticulatus,* which he identified as *Distoma tereticolle* Rudolphi, were: length up to 17 mm.; width, 1.06 mm.; oral sucker, 0.52 mm.; and acetabulum 0.7 mm. There is some confusion here since Manter (1926) (p. 66) reported, "Leidy's *Dist. tereticolle* (from *Esox reticulatus*) also was compared with them (specimens of *D. longum* from the Leidy and Cooper collections), and in the single specimen available in the Leidy collection, the oral sucker, contrary to Leidy's description, was found to be slightly larger than the acetabulum." Stafford (1904) erected the genus *Megadistomum* to contain specimens from *Esox masquinongy* which he regarded as identical with *Distoma longum* of Leidy and distinct from *Azygia tereticollis.* Specimens of *Megadistomum longum* (Leidy, 1851) measured up to 5 inches in length when fully extended and up to 3 mm. in breadth, whereas those identified as *A. tereticollis* measured 12 mm. in length and 1 mm. in width. Stafford reported that the largest specimens of *A. tereticollis* were smaller than immature specimens of *M. longum.* Furthermore, he described worms from the stomachs of *Lota maculosa* and of *Stizostedion vitreum* as members of a new genus and species, *Mimodistomum angusticaudum.*

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Marshall and Gilbert (1905) described *Azygia loossi* from the large-mouth bass, *Micropterus salmoides;* the pike, *Lucius lucius;* and the bowfin, *Amia calva.* The worms contained only a few eggs and obviously were not fully mature. They measured 5 to 7 mm. in length, 0.5 mm. in width; the acetabulum was near the middle and the gonads in the caudal one-sixth of the body.

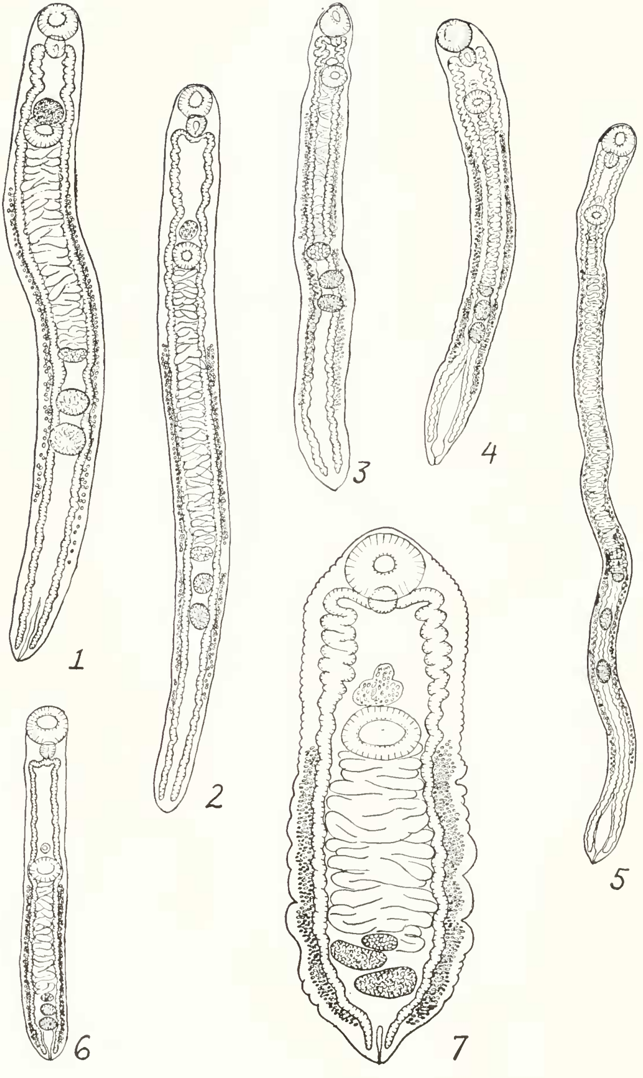
Ward (1910) described *Azygia sebago* from *Salmo sebago* taken at Lake Sebago, Maine. All of seven fishes examined were infected; the worms measured up to 10 mm. in length and from 0.7 to 1.0 mm. in width. From the magnification given, the figured specimen was about 6 mm. long and 0.8 mm. wide. The average diameter of the oral sucker was given as 0.68 mm. and the acetabulum was "distinctly smaller." Specimens presumed to belong to the same species were found in other fishes of Lake Sebago. Two worms were removed from the stomach of a single specimen of *Perca flavescens* and measurements were given for one of them. It was 4.08 mm. long, 0.77 mm. wide; the oral sucker measured 0.51 by 0.57 mm. and the acetabulum 0.35 by 0.40 mm. Four of nine eels, *Anguilla chrysypa* (= *A. rostrata*) were infected with an average of three worms per fish. No descriptive data were given, so presumably they conformed to the specific diagnosis. Eleven of twelve young *Esox reticulatus* were heavily infected; as many as 80 worms were found in a single host. These parasites were more slender and measured 10 to 18 mm. in length. It may be doubted whether they are conspecific with the shorter, more robust worms from the other hosts. Ward reported that smelt, *Osmerus mordax,* were eaten by the larger fishes; the parasites were found also in the stomachs of smelt, although in this host the worms were usually smaller and sexually immature. He noted that the specimens identified by Stafford (1904) as *Azygia tereticolle* are smaller (12 mm. long and 1 mm. wide) than the European species and expressed the belief that they may have been *A. sebago.*

Goldberger (1911) recognized *A. loossi* as a valid species and did not mention *A. sebago,* as Ward's account was probably not available when he wrote his paper. He reported on specimens collected from *Amia calva* taken in Indiana lakes; certain of the worms were identified as *A. lucii,* and others were described as members of two new species, *Azygia bulbosa* and *Azygia acuminata.* Also, he described worms from the stomach of the rock bass, *Ambloplites ruprestris,* as members of a new genus and species, *Hassallius hassalli.*

Odhner (1911) erected the family Azygiidae to contain *Azygia, Otodistomum, Leuceruthrus,* and *Ptychogonimus.* He stated that in the genus *Azygia,* measurements of eggs and extent of vitellaria have little value for specific determination. He declared that *Megadistomum longum* (Leidy, 1851) Stafford, 1904 and *Mimodistomum angusticaudum* Stafford, 1904 are members of the genus *Azygia* and the two generic names were relegated to synonymy. He suggested the probable identity of *A. tereticollis* of America with *A. lucii* of Europe. He criticized Goldberger's work, suppressed *Hassallius* as a synonym of *Azygia,* and expressed the belief that *A. angusticauda, A. loossi, A. acuminata,* and *A. bulbosa* are members of a single species.

As noted, Cooper (1915) described worms which he identified as *A. lucii* from the pike, *Lucius lucius;* the muskellunge, *Lucius masquinongy; Lucioperca* sp.; and immature specimens were recovered from *Salvelinus namaycush* and *Micropterus dolomieu.* He stated that all the worms from the muskellunge are identical with Stafford's *Megadistomum longum* (Leidy) and the smallest one with eggs was 8

Plate I



mm. long. Cooper noted the variable size of worms at the time of egg production. The smallest gravid specimen from the pike was 6 mm. long, but another from the pike, 14 mm. long, was less mature than the one 6 mm. long; others 6 to 14 mm. in length were fully gravid. All the worms from the trout, *S. namaycush,* and the black bass, *M. dolomieu,* including the largest one, 11 mm. long, were immature. Other young and immature specimens from the stomach of *Perca flavescens* were regarded as possible members of this species. Worms from the pickerel (not named) resembled *A. angusticaudum* (Stafford, 1904) but were too contracted to permit positive identification, and others from the pike had a large, globose excretory vesicle, described by Goldberger as characteristic of *A. bulbosa,* but Cooper stated that the shape of the excretory vesicle as well as the length, extent, and "breaking" of the vitellaria are so variable as to be of little use in the delineation of species. Cooper recognized the validity of *A. acuminata,* since 9 specimens from the stomach of *Amia calva* agreed substantially with Goldberger's description of this species.

Ward (1918) stated (p. 392), "Despite many records of its occurrence, the common European *A. lucii* (= *A. tereticolle*) has not been found in North America. Several species peculiar to this continent occur in *Amia calva, Micropterus salmoides* and *dolomieu, Esox lucius* and *reticulatus, Ambloplites ruprestris, Salvelinus namaycush, Lioperca, Lota lota,* and *Salmo sebago.*"

Manter (1926) gave a systematic review of the family Azygiidae; he agreed with Ward in regarding the American specimens as specifically distinct from those of Europe but admitted (p. 57) that "*Azygia* is the only genus of the family showing taxonomic confusion in its species." Accepting the statements of Odhner and Ward, he distinguished *Azygia longa* from *A. lucii* on the extent of the vitellaria, which in the European species are reported not to extend behind the testes, and on the shape of the pharynx, which in *A. lucii* is reportedly cylindrical and twice as long as wide. After detailed study and tabular comparison of morphological features, Manter recognized only three species of *Azygia* in North America, *viz., A. longa* (Leidy, 1851), *A. angusticauda* (Stafford, 1904), and *A. acuminata* Goldberger, 1911. Manter confirmed the suspicion of Odhner (1911) that *Azygia loossi* is identical with *Mimodistomum angusticaudum* Stafford, 1904. As syno-

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PLATE I

FIGURE 1. *Azygia lucii,* from *Esox lucius;* specimen collected and identified by Prof. M. Braun, Königsberg, 7 July 1902; 23 mm. long, ventral view; U. S. National Museum, Helminthological Collection No. 3359.

FIGURE 2. *Azygia lucii,* from *Amia calva;* 20 mm. long, ventral view, (Ward Collection) U. S. N. M., Helminth. Coll. No. 51,403.

FIGURE 3. *Azygia longa,* from *Esox reticulatus,* identified by Albert Hassall; 12.4 mm. long; U. S. N. M., Helm. Coll. No. 49.

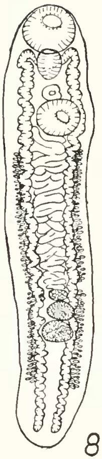
FIGURE 4. *Azygia longa,* from *Esox niger;* 5.2 mm. long, collected 1955 by Paul Krupa, southern New Hampshire.

FIGURE 5. *Azygia longa,* from *Esox niger;* 19 mm. long, collected 1955 by Paul Krupa, southern New Hampshire.

FIGURE 6. *Azygia angusticauda* (type of *Azygia loossi,* Marshall and Gilbert, 1905), from *Micropterus salmoides;* 4.88 mm. long, ventral view; U. S. N. M., Helm. Coll. No. 10,679.

FIGURE 7. *Azygia angusticauda,* from *Stizostedion vitreum;* 10.5 mm. long; ventral view, (Ward Collection) taken by H. W. Manter 4 April 1926, Rock River, Illinois; U. S. N. M., Helm. Coll. No. 51,402.

PLATE II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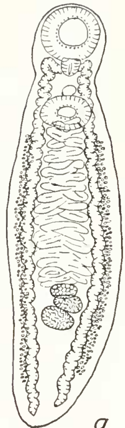
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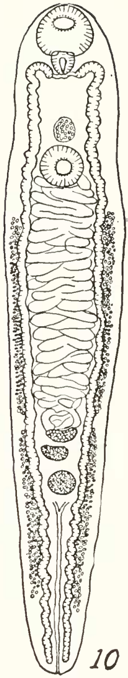
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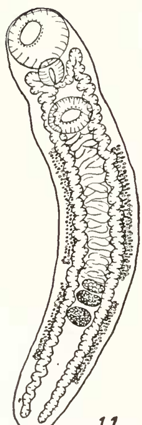
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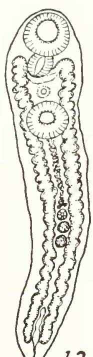
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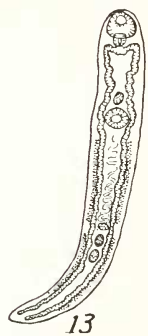
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nyms of *A. longa* (Leidy), Manter listed: *Distomum longum* Leidy, 1851; *Distomum tereticolle* of Leidy, 1851; *Megadistomum longum* (Leidy) of Stafford, 1904; *Azygia tereticolle* of Stafford, 1904; *Azygia sebago* Ward, 1910; *Azygia bulbosa* Goldberger, 1911; *Hassallius hassalli* Goldberger, 1911; and *Azygia lucii* of Cooper, 1915. He discussed the problems of specific determination, noted the bundles of longitudinal muscles which traverse the parenchyma and quoted Leuckart's description of them, and stated that in such elongate and powerfully muscled trematodes, contractions not only alter the general shape of body but the form and relative position of internal organs. Concerning differences in size and sexual maturity, he observed that in the related species, *Otodistomum cestoides,* specimens increase six to seven times in size after attainment of sexual maturity. This fact was used to justify the inclusion in a single species, *A. longa,* of gravid specimens 3.9 mm. long which had been described as *A. bulbosa,* and others which measured up to 3 inches in length and had been described as *A. longum.* It is true that these specimens were from different host species and worms grow larger in larger hosts, but it is doubtful whether host influences can produce such extreme range in size within a single species. Manter's description of *A. longa* was based largely on worms which Ward had described as *A. sebago* and which Manter regarded as identical with *A. longa.* The specific features of *A. sebago* were not clearly defined; there is uncertainty concerning the species, since there is strong probability that material of more than one species was included in the specific diagnosis. According to Manter who studied the Ward collection (p. 64), "*A. sebago* averages about 6 to 8 mm. in length. Specimens were found as small as 1 mm. and no ova were present in forms 2.85 mm. long. . . . Of the other *Azygia* species, *A. bulbosa* Goldberger is most evidently identical with *A. sebago.* Type material of both species was studied. . . . The original type material of *Hassallius hassalli* was also examined for comparison. . . . In fact, after allowance is made for body contraction, this form can not be distinguished from the other common American forms as represented by *A. sebago* and *A. bulbosa.*"

Van Cleave and Mueller (1934) remarked on the variability in fundamental characters, such as the anterior and posterior limits of the vitellaria and the position of the gonads, in the genus *Azygia.* They endorsed the action of Manter in reducing the number of species in North America and went even further in reducing *A. acuminata* to synonymy with *A. longa.* They noted that Manter had listed *A. bulbosa* as a synonym of *A. longa*, and since they regarded *A. acuminata* and *A.*

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PLATE II

FIGURE 8. *Azygia sebago,* from *Perca flavescens,* Sebago Lake, Maine, 1907, 4.26 mm. long, ventral view, (Ward Collection); U. S. N. M., Helm. Coll. No. 51,401.

FIGURE 9. *Azygia acuminata,* from *Amia calva,* Indiana, type of Goldberger, 1911, 6.6 mm. long, ventral view; U. S. N. M., Helm. Coll. No. 10,500.

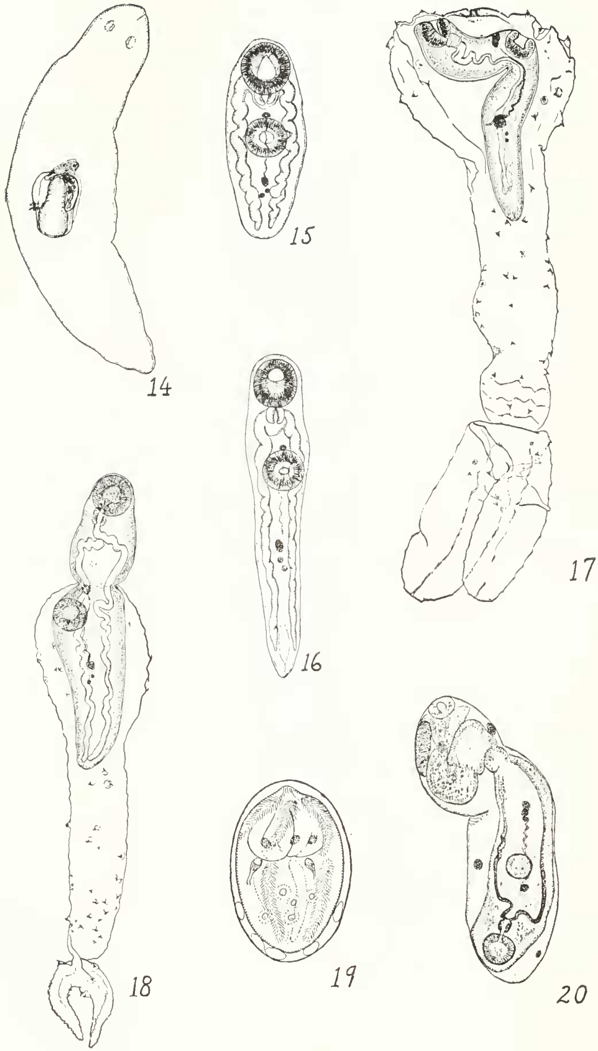
FIGURE 10. *Azygia sebago,* from *Anguilla rostrata,* Falmouth, Mass., 1955, flattened specimen, 12.5 mm. long, ventral view.

FIGURE 11. *Azygia bulbosa,* from *Amia calva,* Indiana, type of Goldberger, 1911, 8.6 mm. long, ventral view; U. S. N. M., Helm. Coll. No. 10502.

FIGURE 12. *Azygia sebago,* from *Anguilla rostrata,* Falmouth, Mass., 1954, immature specimen, 2.66 mm. long, ventral view.

FIGURE 13. *Azygia sebago,* from *Anguilla rostrata,* Falmouth, Mass., 1955, young specimen with 46 eggs in the initial one-half of the uterus, 5.3 mm. long, ventral view.

PLATE III



*bulbosa* as synonyms, *A. acuminata* should also become a synonym of *A. longa*. The reasoning is sound if the postulates are correct, which now appears doubtful. The specimen shown in their Figure 9 (5) which is referred to *A. angusticauda* and the one Figure 9 (7) referred to *A. longa* are so similar that they are probably conspecific and they may not belong to either *A. angusticauda* or *A. longa*. They closely resemble the worms from the eel, identified in this paper as *A. sebago*.

In other surveys of trematode parasites of fishes, *A. angusticauda* and *A. longa* have been reported in eastern North America but *A. longa* may not extend into the area of Lake Huron and northern Wisconsin and neither species has been found in the fishes of western Canada. Lyster (1939) reported *A. longa* from *Esox lucius* and *Anguilla rostrata* in Canada. The single worm from *E. lucius* is probably a young specimen of *A. longa,* but those from eels are very different. He stated that some of them could be assigned to *A. angusticauda* and specimen No. 1 in his table, which is 4.8 mm. wide at the acetabulum, is probably *A. angusticauda*. The others, which are the same length as the one from *E. lucius* but are twice as wide, are very similar to those from eels on Cape Cod. Miller (1940) reported *A. angusticauda* from *Stizostedion vitreum* and *Micropterus dolomieu* in the central St. Lawrence watershed. Miller (1941) restudied the collection of Stafford. He found a specimen from the muskellunge which he identified as *Megadistomum longum;* it was 18.5 mm. long, 1.2 mm. wide, and there are no eggs in the uterus. Another specimen, from *Lota maculosa* and identified as *A. tereticolle,* is 6.5 mm. long, 0.5 mm. wide, and contains eggs. If these worms belong to *A. longa,* as stated, it is difficult to explain the sexual maturity of the smaller individual. In the Stafford collection Miller found two mature and several juvenile specimens of *Mimodistomum angusticaudum*. One of the mature specimens, 7.25 mm. long and 1.65 mm. wide, shown in his Figure 13, is typical, with the acetabulum near the middle and the gonads in the posterior one-sixth of the body. This account of the original Stafford specimens definitely relegates *Azygia loossi* Marshall and Gilbert, 1905 to synonymy with *A. angusticauda* (Stafford, 1904). Choquette (1951) reported both *A. longa* and *A. angusticauda* from the muskellunge, *Esox m. masquinongy*, in the St. Lawrence watershed. Meanwhile, Bangham (1944) examined 1,330 fishes, representing 38 different species, from 40 different locations in northern Wisconsin. He did not find *A. longa,* but *A. angusticauda* was present in 12 species of fish. Bangham and Venard (1946) examined 676 fishes, belonging to 22 species, from Algonquin Park lakes. Worms from *Anguilla rostrata* were

PLATE III

FIGURE 14. *Dugesia tigrinum,* 7 mm. long, experimental infection, two juvenile *Azygia sebago* in the pharyngeal pockets.

FIGURE 15. *Azygia sebago,* 0.81 mm. long, natural infection, from pharyngeal cavity of *D. tigrinum,* juvenile worm found by J. Louis Bouchard.

FIGURE 16. *Azygia sebago,* juvenile worm from *D. tigrinum,* 1.17 mm. long, natural infection, specimen from J. Louis Bouchard.

FIGURE 17. *Azygia sebago,* cercaria, naturally emerged, a fixed and stained specimen.

FIGURE 18. *Azygia sebago,* cercaria, from a crushed snail, larva not entirely mature and only partially enclosed in the enlarged, basal end of the tail, furci shriveled, a fixed and stained specimen.

FIGURE 19. *Azygia sebago,* miracidium in egg, from sketches made of living larvae.

FIGURE 20. *Azygia sebago,* redia in which the pharynx is recognizable; the body of the cercaria is 0.8 mm. long, the furci 0.18 mm. long; fixed and stained specimen.

identified as *A. longa;* others from *Micropterus dolomieu, Perca flavescens* and *Lepomis gibbosus* were identified as *A. angusticauda.* Bangham and Adams (1954) did not find *Azygia* in the examination of 5456 fishes, belonging to 36 different species, taken in the Columbia, Fraser and other rivers of western Canada. In a survey of parasites from 1667 fishes, representing 53 species, from Lake Huron and Manitoulin Island, Bangham (1955) found *A. angusticauda* in the northern channel catfish, *Ictalurus l. lacustris.* This parasite obviously can infect a large number of species of fish.

Knowledge of the life-history of azygiid trematodes dates from the publication by Szidat (1932) on the developmental cycle of *Azygia lucii,* a common parasite in the stomachs of salmonid fishes, especially species of *Esox,* in Europe. Szidat found that the large, furcocercous, cystocercous larva, *Cercaria mirabilis* Braun from *Lymnaea palustris,* when fed to young pike, *Esox lucius,* developed in ten days into adult *Azygia lucii.* He recalled the statement of Looss (1894), that when small pike are eaten by larger ones, the azygiid parasites leave the stomach of the ingested fish and establish themselves on the stomach of the preditor; and stated (p. 501), "Überdies sind ältere Hechte keine Planktonfresser mehr, so dass für sie die Übertragung auf dem zuletzt geschilderten Wege den vorherrschenden Modus darstellen wird, und die jugendlichen Hechte demnach biologisch doch als Zwischen- oder Hilfswirte zu werten sind." Szidat reported that other small fishes also ingest the cercariae and may serve as transport hosts, but in these species the parasites do not develop to sexual maturity. He found juvenile *A. lucii* in the stomachs of small predacious fishes belonging to the genera *Perca, Lucioperca,* and *Gasterosteus.* Szidat described the cercaria-producing generation as a redia, which lacks a digestive tract but in which the pharynx persists as an organ for ingesting fragments of the digestive gland of the snail host and also as a birth pore. He traced the development of the cercariae and noted their resemblance to those of the strigeids and schistosomes. The cercariae are not encysted in the snail host. The body of the cercaria sits in a narrow depression at the anterior end of the flattened tail-stem. The cercariae mature in the haemocoele of the snail and emerge into the mantle cavity. In water, the proximal portion of the tail begins to swell and the body of the larva, anchored in the base of the depression by the tubule of the excretory system, is enveloped by the base of the tail and enclosed in it. Szidat also described *Cercaria splendens,* believed to represent a second species of *Azygia,* but the adult stage and final hosts were not discovered.

The achievement of Szidat in working out the life-cycle of *A. lucii* disclosed that the furcocercous, cystocercous larvae of the Mirabilis type, originally regarded by Leuckart as free-swimming sporocysts and shown by Braun (1891) to be cercariae when he described *Cercaria mirabilis,* are developmental stages of azygiid trematodes. The first member of the group was found by Wright in a fresh-water aquarium and described (1885) as a free-swimming sporocyst. Ward (1916) named the species *Cercaria wrighti* and described a second species, *Cercaria anchoroides,* collected in top and bottom tow every day from July 25 to August 5, 1893, in Lake St. Clair, Michigan. Subsequent investigators have reported other members of the Mirabilis group; sixteen species have been described, but some of them are identical. Several of the named species were described from immature stages, taken from crushed snails, and can not be identified with certainty. Others were described from free-swimming cercariae and the hosts are unknown. Certain of them have proved to be larvae of species in the genus *Proterometra,* erected by Horsfall (1933) to contain *Cercaria macrostoma* Faust, 1918. Reviews of the cystocercous cercariae were published by Horsfall (1934), Smith (1936) and Dickerman (1946). Those with forked tails were designated as furcocystocercous by Le Zotte (1954) who showed that members of the family Bivesiculidae also have larvae of this type.

The second report on the life-cycle of azygiid trematodes was given by Stunkard (1950). Larval distomes had been referred to him for identification in the winter of 1949–1950 by Mr. J. Louis Bouchard, then a graduate student at the University of Oklahoma. The worms had been found in planarians, *Dugesia tigrinum,* received from the Marine Biological Laboratory, Woods Hole, Massachusetts. The structure of the larvae indicated that they were azygiids and study of the life-cycle was begun at the Marine Biological Laboratory in the summer of 1950. Records of the Supply Department of the M. B. L. showed that the planarians sent to the University of Oklahoma had been collected in Morse's Pond in Falmouth. Thirty-eight *D. tigrinum* were collected there on July 10, 1950 and a larval trematode, identical with the specimens sent by Mr. Bouchard, was found in the pharyngeal pockets of two of them. Eight additional worms of natural infection were found in 120 *D. tigrinum* examined. To discover the first intermediate host, different species of mollusks were collected from Morse's Pond and isolated. Furcocercous cercariae of the azygiid type emerged from nine of 246 *Amnicola limosa.* Four planarians, examined under the microscope and known to be uninfected, were placed in a finger-bowl with three specimens of *A. limosa* which were shedding these cercariae. After six days exposure, one to four larvae were found in the pharyngeal cavities of each of the planarians. These larvae were identical with those sent by Mr. Bouchard. The tails, in the bases of which the bodies of the cercariae formerly were enclosed, had completely disappeared. Other planarians were subsequently placed in dishes with infected *A. limosa* and larvae found in their pharyngeal pockets. The larvae may persist for several weeks in *D. tigrinum,* but they do not encyst or grow and it is apparent that the planarians serve merely as paratenic or transport hosts. Attempts to feed the cercariae to goldfish and small perch were not successful; the fish would not take the larvae and when introduced into their mouths, the cercariae were expelled. Planarians infected with cercariae were fed, but the results were uncertain and the short period in which the work could be conducted, led to no further information at that time. It was clear that the larvae belonged to a species of *Azygia,* but specific determination could not be established.

Sillman (1953a) reported that in the vicinity of Ann Arbor, Michigan, the mud pickerel, *Esox vermiculatus,* and the bowfin, *Amia calva,* harbor *Azygia longa.* Eggs of the trematode, containing mature miracidia, were fed to both wild and laboratory-raised *Amnicola limosa.* Cercariae producing rediae were found after 21 days and cercariae emerged 42 days after infection. Cercariae fed to *Esox vermiculatus* developed in 20–30 days into egg-bearing worms. Two of 13,500 *Amnicola limosa* were found naturally infected with cercariae which appeared identical with those in experimentally infected snails.

In a thesis submitted for the Ph.D. degree at the University of Michigan, Sillman (1953b) gave further information. He stated that two species of *Azygia* are present in the Ann Arbor area. One species, which he identified as *A. longa,* occurs in both *Esox vermiculatus* and *Amia calva.* The other species, which he identified as *A. acuminata*, was found only in *Amia calva*. Worms assigned to *A. longa* were somewhat longer, more slender and the suckers were slightly smaller than those of *A. acuminata*, but the measurements of worms and organs overlapped. According to Sillman, the collecting ducts of the excretory system branch from the vesicle behind the testes in *A. longa* and between the testes in *A. acuminata*. Although there was much variation, the average size of eggs in *A. longa* was 55 by 31 microns whereas that of *A. acuminata* was 69 by 38 microns. Furthermore, specimens of *Amnicola limosa* did not become infected when fed eggs of *A. acuminata*.

Investigation of the life-cycle and development of *Azygia* has been continued at the Marine Biological Laboratory, Woods Hole, Mass., during the summer months since 1950. An abstract of the results was presented (Stunkard, 1955). Infected snails were found each year and the morphology of the young distome, especially the details of the excretory system, was studied. Hundreds of fishes, including *Esox niger, Perca flavescens, Morone americanus, Micropterus salmoides, Micropterus dolomieu*, and others, were examined in the attempt to find the sexually mature stage of the parasite. The first to be discovered, a small, immature specimen of *Azygia* (Fig. 12) was found in the stomach of an eel, *Anguilla rostrata*, late in the summer of 1954. During the summer of 1955, 42 eels were examined; 10 of them were infected and many fully mature worms were collected. Continued examination of other fishes, especially the pickerel, *Esox niger*, from the same ponds where the infected eels were taken, has not disclosed infection by members of the genus *Azygia*, and it appears that the eel is the natural and possibly the only host for the species in the Woods Hole region. The larger ponds in the area are under the control of the Division of Fisheries and Game, Bureau of Wildlife Research and Management of the State of Massachusetts, and many of them have been stocked with game fishes from time to time. Through the kind cooperation of Mr. Russell Cookingham, a large number of fishes, belonging to various species, were provided during the summer of 1955, when certain of these ponds were inspected to determine their productivity.

Specific determination of the parasites from the eel has proved difficult. Descriptions are wholly unsatisfactory and accordingly, specimens of *Azygia* in the U. S. National Museum were borrowed through the kindness of Dr. E. W. Price and Mr. Allen McIntosh. The material consisted of 6 specimens in alcohol (bottle M 248-D), the type specimens of *Distomum longum* Leidy, and other specimens mounted on slides and bearing the following labels, U. S. National Museum, Helminthological Collection:

No. 49. *Distomum longum* from *Esox reticulatus*, determined by Albert Hassall; 1 slide. (Plate I, Fig. 3.)

No. 3359. *Azygia lucii* from *Esox lucius*, collected and determined by Professor M. Braun, 7 July 1902, Königsberg, Germany; 1 slide. (Plate I, Fig. 1.)

No. 10500. *Azygia acuminata* from *Amia calva*, type and paratypes; 4 slides. (Plate II, Fig. 9.)

No. 10502. *Azygia bulbosa* from *Amia calva*, type and paratypes; 3 slides. (Plate II, Fig. 11.)

No. 10679. *Azygia loossi* from *Micropterus salmoides*, cotypes; 3 slides. (Plate I, Fig. 6.)

No. 51399. *Azygia sebago* from *Salmo sebago,* 5.2 mm. long, 1 slide, H. B. Ward collection.

No. 51401. *Azygia sebago* from *Perca flavescens,* H. B. Ward collection; 2 slides. (Plate II, Fig. 8.)

No. 51403. *Azygia sebago* from *Amia calva,* 20 mm. long, 1 slide, H. B. Ward collection. (Plate I, Fig. 2.)

No. 51402. *Azygia angusticauda* from *Stizostedion vitreum,* collected by H. W. Manter, 4 April 1926, Rock River, Illinois, 2 slides, H. B. Ward collection. (Plate I, Fig. 7.)

Examination of the specimen of *Azygia lucii,* No. 3359 in the U. S. National Museum, invalidates the criteria used by Ward and Manter to distinguish between *A. longa* and *A. lucii.* In this specimen (Fig. 1) which measures 23 mm. in length, collected and identified by Professor M. Braun, the pharynx is not twice as long as broad; in fact, the organ measures 0.80 mm. long and 0.60 mm. wide. Furthermore, the vitellaria extend far behind the posterior testis; the follicles on the left side about one-half the distance from the testis to the end of the body. In the Ward collection there is a specimen from *Amia calva* (Fig. 2) which measures 20 mm. in length and which resembles the European specimen so closely that I am disposed to regard the two as specifically identical. Leidy, Stafford and Cooper all reported the finding of *A. lucii* and it appears that this species does occur in North America. *Esox lucius,* the type host, is circumpolar in range, and the distribution of its parasites may be expected to parallel that of the host. The dispersal of fishes in the northern hemisphere following the last glacial period has been traced by Walters (1955).

Although the criteria used by Ward and Manter to distinguish *A. longa* from *A. lucii* are inadequate, the two forms are probably distinct. About 100 specimens collected by Mr. Paul Krupa from *Esox niger* in southern New Hampshire during the summer of 1955 are so similar to the six worms in alcohol, now in the U. S. National Museum, which constitute the original material of the species described by Leidy (1851) as *D. longum,* that they must be regarded as identical. A representative example from the Krupa collection is shown (Fig. 5) and a smaller one (Fig. 4). These worms are very slender. The Krupa specimens were dropped in cold Duboscq-Brasil fluid and fixed without narcotization or pressure. Oviferous specimens vary from 4 to 26 mm. in length and 1.1 mm. is the greatest width. The width does not increase very much as the worms grow in length. Comparison of Figure 5 with that of *A. lucii* (Fig. 1) portrays what are believed to be specific differences. Further evidence that *A. longa* is distinct from *A. lucii* is afforded by comparison of the cercariae. *Cercaria mirabilis* Braun, 1891, shown by Szidat (1932) to be the larval stage of *A. lucii,* is very different from the cercaria described by Sillman as the larval stage of *A. longa.* Moreover, in Europe *A. lucii* uses a pulmonate snail, *Lymnaea palustris corvus,* as the first intermediate host, whereas according to Sillman, the asexual stages of *A. longa* occur in the pectinibranchiate snail, *Amnicola limosa.*

Recognition of two distinct species, *A. lucii* and *A. longa,* may resolve certain difficulties and clear up confusion in the literature. The worms from *Esox masquinongy* which Stafford (1904) described as *Megadistomum longum* (Leidy) measured up to five inches in length when extended and probably were not identical with *A. longa* of Leidy. Stafford reported a specimen 18 mm. long which contained no eggs. Cooper (1915) identified specimens from *E. masquinongy,* which he regarded as identical with those of Stafford, and others from *E. lucius,* as *Azygia lucii.* His specimens from the muskellunge measured 21 to 48 mm. in length and 1.40 to 2.40 mm. in width, whereas those from the pike were 14 to 20 mm. in length and 0.74 to 1.42 mm. in width. Comparison of the small worms from *E. masquinongy* with worms from *E. lucius* led Cooper to regard them as conspecific. But he was unable to account for the variable size at which eggs are produced in different individuals. He reported that a specimen 14 mm. long from *E. lucius* was less mature than another 6 mm. long from the same host species, and that worms from the trout and small-mouthed black bass were all immature although one from *S. namaycush* was 11 mm. long. Discussing the effect of season on sexual maturity, Manter (1926) wrote (p. 67.) ". . . it is certain that what is evidently the same species does not attain sexual maturity at the same time in different hosts in which it occurs. Thus, while average sized forms are producing eggs in such hosts as pike, pickerel, and salmon, specimens fully as large are still sexually immature in such hosts as smelt, trout, small mouthed black bass, and perch." Admittedly, members of a trematode species attain a greater size in a larger host species, and *E. masquinongy* is much larger than *E. lucius,* but present information strongly indicates that *A. longa* is distinct from *A. lucii,* if, indeed, the large American species is actually *A. lucii* of European fishes.

All previous authors have agreed on the identity of *A. angusticauda* (Stafford, 1904) and *A. loossi* Marshall and Gilbert, 1905. A cotype specimen of *A. loossi* (U. S. Nat. Mus., 10,679), shown in Figure 6, is 4.88 mm. long and is obviously young, with only a few eggs in the uterus. A fully mature, gravid specimen (U. S. Nat. Mus., No. 51,402) from the walleye, *Stizostedion vitreum,* collected by Manter in 1926, which measures 10.5 mm. in length, is shown in Figure 7. In both, the acetabulum is near the middle and the gonads are situated in the caudal one-sixth of the body. The distinctness of this species appears to be well established.

The specimens of *Azygia* found in the eel at Woods Hole are clearly distinct from *A. angusticauda* and, as noted, are probably distinct from *A. longa.* Specimens of *A. longa* are slender and much elongate; those from the eel are shorter and more robust. The worms collected by Mr. Krupa from *Esox niger* in New Hampshire and identified as *A. longa* remained well extended when dropped into Duboscq-Brasil killing fluid, whereas those from the eel contracted strongly with the result that the length was only 6 to 8 mm., less than one-half that of *A. longa.* Accordingly, most of the worms from the eel were killed and fixed under pressure, which resulted in longer, wider, and flatter specimens. The size of the suckers increased as a result of the compression but comparison of Figures 10 and 13, which were made from one of the largest and one of the smallest oviferous specimens, with Figures 5 and 4, of comparable specimens of *A. longa,* portrays differences between the two forms which are believed to be specific.

Whereas the worms from the eel differ distinctly from those identified as *A. longa,* they agree almost completely with Goldberger's description of *A. acuminata* and agree almost as well with the descriptions of *A. sebago* as given by Ward and Manter. Certain worms from the eel are very similar to specimens in the Ward collection labelled *A. sebago.* It is probable that Ward had more than one species and that his description of *A. sebago* was based on specimens of both *A. longa* and

*A. sebago.* The worm from the Ward collection which bears the U. S. Nat. Mus., No. 51,401, shown in Figure 8, is clearly *A. sebago,* and the worm on U. S. Nat. Mus., No. 51,403, from *Amia calva* shown in Figure 2, is so like *A. lucii* (*cf.* Fig. 1), that the two might be regarded as specifically identical. Other specimens of *A. sebago* agree so completely with Goldberger's description of *A. acuminata* (compare Figs. 8 and 9), that I am inclined to regard them as identical. Since Ward probably confused two species in his description of *A. sebago,* the removal of the elongate specimens leaves the description virtually the same as that of *A. acuminata.* Specific determination may be impossible on the basis of adult morphology alone and knowledge of life-cycles and larval stages may be required to finally solve the problem. Why the species occurs only in *Anguilla rostrata* in the Woods Hole area is quite unknown. The larval stages are relatively abundant in the snails of the region, but sexually mature worms have so far been found only in the eel. The chain pickerel, *Esox niger,* is common in these ponds where it has been introduced in stocking operations. Since the worms develop in eels in ponds where pickerel, perch, bass and other fishes are not infected, it appears either that the ecological conditions and food-chain lead to the infection of eels rather than other fishes or else the other fishes do not retain the parasites. In the latter event, a separate species must be involved.

When worms were removed from the stomachs of eels and placed in pond water, the eggs in the terminal coils of the uterus were extruded in a string of mucus. These eggs appeared to be fully embryonated and the miracidium was studied in the egg. Although active, the larvae did not emerge in water and hatching occurred only after the eggs were ingested by the snail host. Empty shells were recovered in the feces of *Amnicola limosa* that had eaten the eggs. Some of these snails were found later to be infected but since they had been collected from locations where previous exposure to infection was liable, it would be difficult if not impossible to distinguish between a natural infection acquired before collection and an experimental one. But the snails laid eggs in the finger bowls and young laboratory-raised specimens were fed eggs of the parasite. These small snails became infected and although emerged cercariae were not obtained before the end of the summer, the developmental stages in these experimental infections were indistinguishable from comparable stages in natural infections. In nature, the eggs of the parasite are passed in mucous material from the intestines of eels and settle on vegetation and on the slimy surfaces of submerged rocks and sticks. The snails rasp these surfaces for the diatoms which form a major constituent of their food and incidentally ingest the eggs. The larvae remain alive for long periods and since the eggs do not hatch until they are eaten, the probability of reaching a suitable host and continuing the life-cycle is much enhanced. The larvae emerge in the intestine of the snail and bore through the wall to reach the haemocoele, where they become sporocysts. Young sporocysts have been found adjacent to the intestinal wall two weeks after eggs of the parasite were added to the finger bowl with the young snails. Older infections with rediae and developing cercariae were found later, which definitely link the experimental and natural infections. However, the rate of development of the parasites and the degree of maturity of the infection are not regarded as significant. It is common knowledge that asexual stages of digenetic trematodes persist but fail to grow or reproduce if the hosts are not fed. Thus, infections overwinter in a quiescent stage in mollusks that are dormant or in which metabolism is reduced to a low level. In the present instance, although various methods, including those recommended by Moore *et al.* (1953) and by Sandground and Moore (1955) for the rearing of related snails, were employed, it was obvious that the snails, although most of them remained alive, were not properly nourished, did not grow normally, and the tissues had the atrophic appearance typical of inanition.

Cercariae from natural infections were snapped up by guppies and by small bluegill sunfish, *Lepomis macrochirus*, 2 to 4 cm. in length. The young worms were recovered from the stomachs of these sunfish two and three weeks after they were eaten, but there was very little development of the parasites. These small fishes also ate planarians, *Dugesia tigrinum;* so in nature the fishes could contract the infection by eating either the cercariae or infected planarians. The tails of the cercariae cease to beat after about 48 hours and they would then not be attractive to fishes; moreover the larvae die during the next 48 hours. As stated earlier, the young worms live for weeks in the pharyngeal pockets of *D. tigrinum* and this accessory method of employing an additional paratenic or transfer host enhances the likelihood of survival and aids in the completion of the life-cycle. The cercariae are probably not eaten by eels which are at the end of the food-chain that leads to their infection.

## Description of Stages in the Life-Cycle

### *Adult*

The worms are only slightly flattened, almost cylindrical, with rounded ends and enormously developed musculature. Because of the ability to extend and retract the entire body or particular regions to an extraordinary degree, measurements of length and width and location of individual organs have limited significance. A specimen may extend to four or five times its length when contracted, and contraction of different regions can make distances between organs so variable that measurements may be very misleading. Ward (1918) wrote (p. 392), "*Azygia* is a powerfully muscular type and is usually much distorted in the process of preservation so that a lot of specimens taken from the same host at the same time present marked external differences in the preserved condition. Such extreme specimens have been the basis for various new genera, *e.g., Megadistomum* of Leidy and Stafford, *Mimodistomum* of Leidy (*sic*) and *Hassallius* of Goldberger. This same factor has lead to the separation of too many as species." Oviferous specimens from the eel, fixed by the shaking method of Looss, are 3 to 9 mm. long and when fixed under pressure measure 4 to 12.5 mm. in length. Because of the variations caused by muscular contractions on the shape of the body and location of organs, dimensions of the suckers and gonads provide the most reliable morphological data, but these organs appear larger in specimens that have been fixed under heavy pressure. Egg sizes vary too much to provide reliable specific criteria. The worms continue to grow after sexual maturity. A large one and a small one are shown in Figures 10 and 13; both were fixed under pressure and are therefore comparable. Measurements in millimeters of the larger one are: length, 12.5; width, 2.2; oral sucker, 0.96; acetabulum, 0.8; pharynx, 0.36 long and 0.32 wide; ovary, 0.54 by 0.23; anterior testis, 0.5 by 0.33; posterior testis, 0.5 by 0.4. Corresponding measurements of the smaller worm are: length, 5.3; width

0.65; oral sucker, 0.41; acetabulum, 0.34; pharynx, 0.19 long and 0.16 wide; ovary, 0.195 by 0.12; anterior testis, 0.195 by 0.143; posterior testis, 0.24 by 0.16. The eggs, alive, averaged 0.06 by 0.034 mm.; under oil immersion and slight pressure, to study the miracidium, they were slightly larger; in fixed and stained worms they were smaller, and averaged 0.055 by 0.030 mm. In such mounted specimens the eggs are usually collapsed and distorted.

*Miracidium*

The miracidium of *Azygia lucii* was described by von Nordmann (1832), Schauinsland (1883) and Looss (1894) and that of *Azygia acuminata* (possibly a synonym of *A. sebago*) by Manter (1926). The miracidium of the worms identified as *A. sebago,* studied alive in the egg (Fig. 19) and in stained sections of gravid worms, is similar to that of related genera in the family Azygiidae, as reviewed by Manter (1926). Like the others, it lacks cilia and is provided with bristle plates or plaques. It almost fills the egg-shell; the anterior end may be protruded as a conical papilla on which the ducts of the secretory cells open. Radiating from this area, there are five plates or plaques that bear fine bristles arranged in a chevron-like pattern. The anterior ends of the plates are separated by short intervals, which become wider posteriorly. The plaques extend backward about one-third of the length of the larva; the bristles on the anterior portions are larger and longer than those more posteriad. From a naked area at the posterior end of the larva, four bristle-bearing bands extend forward past the middle of the body. The bands are equidistant from each other and both the anterior and posterior ones manifest a spiral tendency, but this aspect may be the result of rotation of the larva within the shell. The appearance of the miracidium is almost identical with that of *Proterometra macrostoma* as reported by Hussey (1945). Hussey described a structure, designated by earlier authors as a "primitive gut", with four nuclei arranged in a linear series. In *A. sebago,* the corresponding structure, which is glandular and probably serves in penetration, consists of four cells which lie side by side rather than in linear series. These cells are disposed as reported by Manter (1926) for the miracidia of *Otodistomum cestoides, Otodistomum veliporum* and *Azygia acuminata.* Manter reviewed previous accounts and presented a strong argument that the organ is not a primitive gut, but a group of unicellular glands. Immediately posterior to the glandular organ there is a bilobed "brain" and the region behind it contains several large, germinal cells. On either side, near the middle of the body, there is a single flame cell from which an excretory tubule leads caudad, but the ducts were not traced to the pores.

*Asexual generations*

The youngest sporocyst was recovered from a loose network of connective tissue on the somatic side of the intestinal wall of a laboratory-raised snail that had been exposed 12 days previously. It was oval, 0.094 by 0.062 mm., with no lumen; it contained germinal cells but no germ-balls (embryos). Other larger sporocysts were found in older infections; one, 0.126 by 0.08 mm., contained germinal cells and 6 small germ-balls; another, 0.189 by 0.12 mm., contained germinal cells and 9 germ-balls of varying sizes. In a snail killed one month after exposure, the mother sporocyst could not be recognized but there were 26 rediae scattered about in the haemocoele. The smallest was 0.25 by 0.18 mm., and in addition to germinal cells it had four small spherical to oval germ-balls, 0.02 to 0.04 mm. in diameter. A redia with larger germ-balls but no recognizable cercariae measured 0.57 mm. long and 0.18 mm. wide; the pharynx was 0.08 mm. in diameter and there was a sac-like gut, 0.11 mm. long and 0.032 mm. wide. The largest redia was 1.3 by 0.3 mm. and in addition to smaller embryos, it contained two cercariae, one of which was more than half-grown and had small furci. Whether or not there is a second generation of rediae was not determined.

The cercaria-producing generation of species in the genus *Azygia* was recognized by Szidat (1932) as redial, although the pharynx undergoes reduction to a mere vestige and the intestine completely disintegrates. As noted by Szidat in *A. lucii,* the pharynx, which he termed "rudimentary", serves for the ingestion of bits of the digestive gland of the host and persists as a birth-pore through which the cercariae emerge. The small rediae are vermiform and very active; the pharyngeal end may be inrolled and then everted, while the opposite end may be protruded as a pointed, tail-like structure. Older rediae may extend to a length of 3 mm. and on contraction of the circular muscles, present an annulate appearance. On contraction of the longitudinal muscles they become oval and about 1 mm. in width. The one shown in Figure 20 is bent and as mounted measures 1.12 by 0.325 mm.; in it the pharynx is still distinct. The older, larger, rediae have little mobility but pulsations of one and sometimes two can occasionally be seen through the shell of an infected snail. The number of cercariae in a redia is small; often there is only one and rarely are there more than three recognizable cercariae; other individuals are still in the germ-ball stage, together with a few germinal cells attached to the body wall, chiefly at the posterior end of the redia. Apparently the development of one cercaria restrains the development of others. An infected snail may liberate one or two cercariae each day for a few days and then none for a week or more. The large size of the cercariae is correlated with the slow development and the small number produced.

### *Cercaria*

Developing cercariae are typical furcocercous larvae. As the embryo reaches a length of approximately 0.25 mm., a constriction appears and gradually separates the posterior one-fourth to one-third of the larva as an oval, tail-rudiment. At about this stage, the oral sucker is faintly outlined. When the larva has reached a length of 0.4 to 0.5 mm., the suckers are distinct, the acetabulum is in the posterior half of the body, the tail is about three-eighths of the total length, and the furcal buds are beginning to appear. As development proceeds, the tail increases in length more rapidly than the body; its basal portion, about one-sixth of its length, begins to enlarge and by the time the gonads are recognizable, the anterior end of the tail forms a cup-like ring (Fig. 20), at the base of which the constricted caudal end of the distome is continuous with the tissues of the tail. The cercariae complete their growth in the rediae and emerge into the haemocoele of the snail. While studying the excretory pattern of a redia which was under some pressure, an immature cercaria emerged, tail first, through the old pharyngeal opening. The cercariae mature in the haemal sinuses of the snail, especially the branchial sinus, and emerge through the respiratory opening. During growth, the basal portion of the tail is much enlarged by the accumulation of spongy, fibro-elastic, alveolar tissue which, when the cercaria emerges from the snail, absorbs water and expands rapidly. As a result, this portion of the tail extends forward, encapsulating the body of the cercaria. If infected snails are crushed and immature larvae are liberated into water, the base of the tail is unable to completely engulf the body of the larva (Fig. 18).

Mature, normally emerged cercariae measure 1.8 to 2.3 mm. in length. The expanded, basal portion of the tail is flattened, 0.5 to 0.75 mm. in width, and slightly more in length. The stem of the tail, that portion from the spongy, rigid, basal part to the furci, is 1.0 to 1.5 mm. in length and 0.26 to 0.46 mm. in width. It tapers slightly from the basal to the distal end. It is distinctly flattened and set at right angles to the dorso ventrally flattened body of the larva, so that when looking at the flat aspect of the tail, the body appears in lateral view (Fig. 17). This stem portion of the tail consists of two bands of longitudinal muscles, one on each of the flat surfaces. These muscles are attached at one end to the rigid, spongy portion of the tail and at the other end to the bases of the furci. The furci are flattened, 0.55 to 0.90 mm. in length and 0.20 to 0.28 mm. in width. Normally they are held almost at right angles to the tail stem, whose muscle bands contract alternately, so that the flapping of the tail from side to side produces a sculling effect that pulls the larva through the water. After the beat of the tail is unable to lift the larva from the bottom, it continues for a day or two and this flapping motion makes the larva an attractive lure for small fishes and perhaps other predators. The basal end of the tail becomes sticky and may lightly attach the larva to the substratum. How the larvae reach the pharyngeal cavity of the planarians is not clear. The body is firmly enclosed in the chamber at the anterior end of the tail and could be liberated only by dissolution of the tail. According to Hyman (1951, p. 107), "The triclads do not swallow their food whole but suck it in by peristaltic action of the protruded pharynx." and (p. 199), "The Turbellaria are as a class carnivorous. . . . Favorite items of food of the smaller species are rotifers, copepods, cladocerans, nematodes, annelid worms, etc.,". Perhaps the planarian seizes the larva, and as the tail is sucked in and digested, the young worm is liberated and attaches to the external surface of the pharynx, whence it is carried into the cavity when the pharynx is retracted.

The tail bears many papillae, scattered somewhat irregularly over the surface except for the distal three-fourths of the furci. Each is about 0.05 mm. in diameter, 0.025 mm. tall, and is surmounted by a recurved hook, 0.012 to 0.015 mm. in length. The tail also has many opaque patches, which on higher magnification are seen to consist of minute spherules. The excretory system of the larval body is continuous with that of the tail and the constricted caudal end of the body contains the common excretory canal which traverses the stem of the tail, bifurcates at the bases of the furci, and the resulting tubules open at the tips of the furci. The pattern of flame cells in the tail was not resolved.

The morphology of the young worm, released from the chamber in the tail, is typically azygiid (Figs. 15, 16). The cuticula is unarmed but the preacetabular region bears many papillae and a bristle has been observed at the tip of certain of them. There are at least a dozen papillae, 0.018 to 0.020 mm. in diameter, around the anterior end of the worm. Living specimens vary from 0.7 to 1.3 mm. in length and 0.16 to 0.28 mm. in width. The acetabulum varies from 0.10 to 0.13 mm., and the oral sucker, 0.11 to 0.14 mm., in diameter. The pharynx measures 0.05

to 0.07 mm. in length and usually slightly less in width. The digestive ceca are filled with yellow material, derived from the digestive gland of the snail. The excretory system is complex but has been worked out completely. The pore is terminal and a common duct leads forward almost to the level of the testes. The posterior one-half of this duct may expand to form a bladder-like enlargement, or if the pore is blocked and fluid accumulates, the enlargement may extend farther forward. Behind the testes the common duct divides, forming two ducts which pass forward, median to the digestive ceca. As the ceca turn mediad to join the pharynx, the excretory ducts pass below them and continue on either side of the oral sucker almost to the anterior end of the body. There is, however, no connection between the ducts of the two sides. Anterolateral to the oral sucker, the duct of each side doubles backward and continues posteriad, giving off eleven branches. Each branch divides three times, forming two primary, four secondary and eight tertiary branches. Each tertiary branch receives the capillaries from four flame cells. The flame cell formula accordingly is $2\ (11 \times 32)$ or 704 flame cells in the body. This observation is in agreement with that of Looss (1894) who described the same pattern in *Azygia tereticolle* (= *A. lucii*). He regarded the ascending portions of the excretory system as parts of the excretory vesicle and the descending limb with its branches as the collecting ducts. He suggested the possibility of variation in the number of branches and of anastomoses between collecting ducts; however, I have found a constant number of branches and the apparent anastomoses can be resolved as places where one duct crosses another. Counting backward from the anterior end of the body, the first side branch is located at the level of the oral sucker; the second is at the level of the bifurcation of the digestive tract, *i.e.,* the posterior end of the pharynx; the third branch is anterior to the acetabulum; the fourth is at the middle of the acetabulum; the fifth is at the level of the posterior end of the acetabulum; the sixth and seventh are close together a short distance behind the acetabulum; the eighth, ninth and tenth are almost equally spaced; while the eleventh and last, which is the terminal group of the recurrent limb, is distributed to the extreme posterior end of the body around the excretory bladder. The reproductive organs are represented by groups of deeply staining cells, shown in Figures 15, 16 and 17.

## Summary

A chronological account of the genus *Azygia* discloses discordant observations and divergent opinions. Dawes (1946) recognized only a single species, *A. lucii*, in Europe. In it he included *A. robusta* Odhner, 1911, which reaches a length of 47 mm. and *Ptychogonimus volgensis* von Linstow, 1907, which measures 5 to 6 mm. in length and had been transferred to *Azygia* as a valid species by Odhner (1911). In America several species have been described, but there is no agreement on the number that are distinct and valid. In fact, there is no adequate information on the extent of variation that occurs in a natural species, and consequently on the features that can be relied on to distinguished between species. This situation is not peculiar to *Azygia,* but obtains in many genera. It is the natural result of development by members of a parasitic species in different hosts, invertebrate and vertebrate, often of different taxonomic groups, which differ in their nutritional and other physiological conditions, and accordingly influence the development and

morphological features of the parasite. Until the life-cycle is known and the variation that normally occurs in each possible host is measured, the precise limits of specificity will remain uncertain. Comparison of specimens and descriptions indicates that *A. lucii* may be endemic in North America, that possibly it is distinct from *A. longa* (Leidy), that *A. angusticauda* (Stafford) is a valid species, and that the species described by Goldberger (1911) may be identical with *A. sebago* Ward. Information concerning the life-history of species in the genus *Azygia* is meager. Szidat (1932) showed that *Cercaria mirabilis* Braun is the larva of *A. lucii*. He described a second larva, *Cercaria splendens*, presumably another species of *Azygia*, but the adult stage remains unknown. Sillman (1953a) reported the life-cycle of a species that he identified as *A. longa* and the present paper presents data on the morphology and life-history of a species believed to be *A. sebago*. Stages in the cycle are described and figured.

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